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Effect of surgical delay on bacterial colonization in proximal humeral fractures

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The effect of surgical delay on bacterial colonization in proximal humeral fracture

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42 All the authors state that they have read and approved the final submitted manuscript and that
43 they have contributed substantially to this paper according to research design, acquisition,
44 analysis, interpretation of data, drafting the paper or revising it critically, approval of the
45 submitted and final versions.

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47 Running title: bacterial colonization in humeral fractures

48

Abstract

Introduction: Postoperative infection is a severe complication after proximal humeral fracture surgical treatment. The aim of this study was to determine if the surgical delay could modify the number and type of bacteria on the surgical site.

Materials and methods: A two stages study was set up. In the first stage the effect of delay was simulated in 20 patients affected by proximal humeral fracture treated conservatively. In a second stage, the effect of delay was measured in 20 patients that underwent surgery. In stage 1, three skin culture swabs were taken in correspondence of the deltopectoral approach, the day of the fracture(day 0), the day after(day 1) and five days after fracture(day 5). In stage 2, skin swab cultures were taken the day of trauma and immediately before surgery, cultured on various media suitable for aerobic and anaerobic bacteria.

Results: The number of bacteria increased over the course of the study, from day 0 to day 5, both considering the total number of Colony-Forming Unit and individual species of pathogen bacteria. The second stage of the study confirmed these data. An increasing number of bacteria was observed in patients that underwent surgery later than 2 days from trauma.

Conclusions: The delay to surgery increased bacterial colonization of the skin in the deltopectoral approach area including common pathogenic bacteria such as *Staphylococcus aureus*, coagulase-negative staphylococci and *Propionibacterium acnes*. This might justify the correlation between delay to surgery and risk of infection.

Keywords: proximal humeral fracture, infection, propionibacterium acnes, skin swab cultures, delay of surgery.

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Introduction:

Proximal humeral fractures (PHF) are one of the most common fractures as they represent 45% of the fractures of the upper limb¹. Considering the increasing age of the population, the incidence of proximal humeral fractures is likely to increase². Approximately 20% of these fractures require surgery, with the aim of restoring function and resolving pain³. Despite the good outcomes that have been reported after surgery, high complication rates have been described⁴⁻⁷. Postoperative infection is one of the most severe complications. The incidence varies from 0 to 8% depending on the studies, and the consequences can be devastating for function, patient quality of life and total costs^{8,9}.

Unlike the obvious relevance of this topic, very few articles have focused on the risk factors for the development of infection after surgical treatment for proximal humeral fractures. A recent multicenter study by Blonna *et al.* showed a potential correlation between acute infection and delayed surgery with a peak of infection at around 5 days post-trauma¹⁰.

Although the explanation of this observation remains unclear, one of the hypotheses is that the delay to surgery determines an increasing number of bacteria on the surgical site. Shoulders affected by proximal humeral fracture have in fact some peculiarities that distinguish them from elective surgery. In the case of a fracture, patients commonly undergo surgery after some days of immobilization with a bandage or a sling, often without the possibility of washing the affected shoulder. This might determine an increase in the number of skin bacteria that could potentially affect the surgical site.

The objective of our study was to determine if the delay to surgery could modify the skin bacterial load and type on the surgical site.

93 **Materials and Methods:**

94 In light of the Italian law, no institutional review board approval was mandatory for this study.

95 The study has been performed in accordance with the ethical standards in the 1964

96 Declaration of Helsinki and has been carried out in accordance with relevant regulations of

97 the Italian National Health care System. An informed consent was obtained for all patients.

98 This study was set up in two stages. In the first stage, our hypothesis was tested in a model

99 simulating the conditions of a proximal humeral fracture treated surgically. This model was

100 designed to better control some of the variables such as delay to surgery and patients-related

101 variables. In a subsequent stage our hypothesis was tested directly in patients that underwent

102 surgery for treatment of proximal humeral fractures.

103 First stage.

104 Twenty-five consecutive patients affected by proximal humeral fracture were initially

105 included in this study. All the patients were recruited in the Emergency Room of the

106 Department of Orthopaedics and Traumatology, Mauriziano-Umberto I Hospital, University

107 of Torino, the day of the trauma. The inclusion criteria were: a) consent to the study protocol,

108 b) undisplaced proximal humeral fracture with indication to a conservative treatment. After

109 inclusion in the study, patients were interviewed for comorbidities, demographic data were

110 collected, and the first skin culture swab was taken from the area of the deltopectoral

111 approach (day 0). The patients were managed in a way that simulated the management of

112 patients treated surgically. In patients planned for surgery the affected shoulder is placed in a

113 bandage for pain control and the patient is discharged at home until the day of surgery. In the

114 first stage of the study, since the patients were treated conservatively, the delay of surgery was

115 simulated by assessing the same patient for an ambulatory visit in two different dates. The

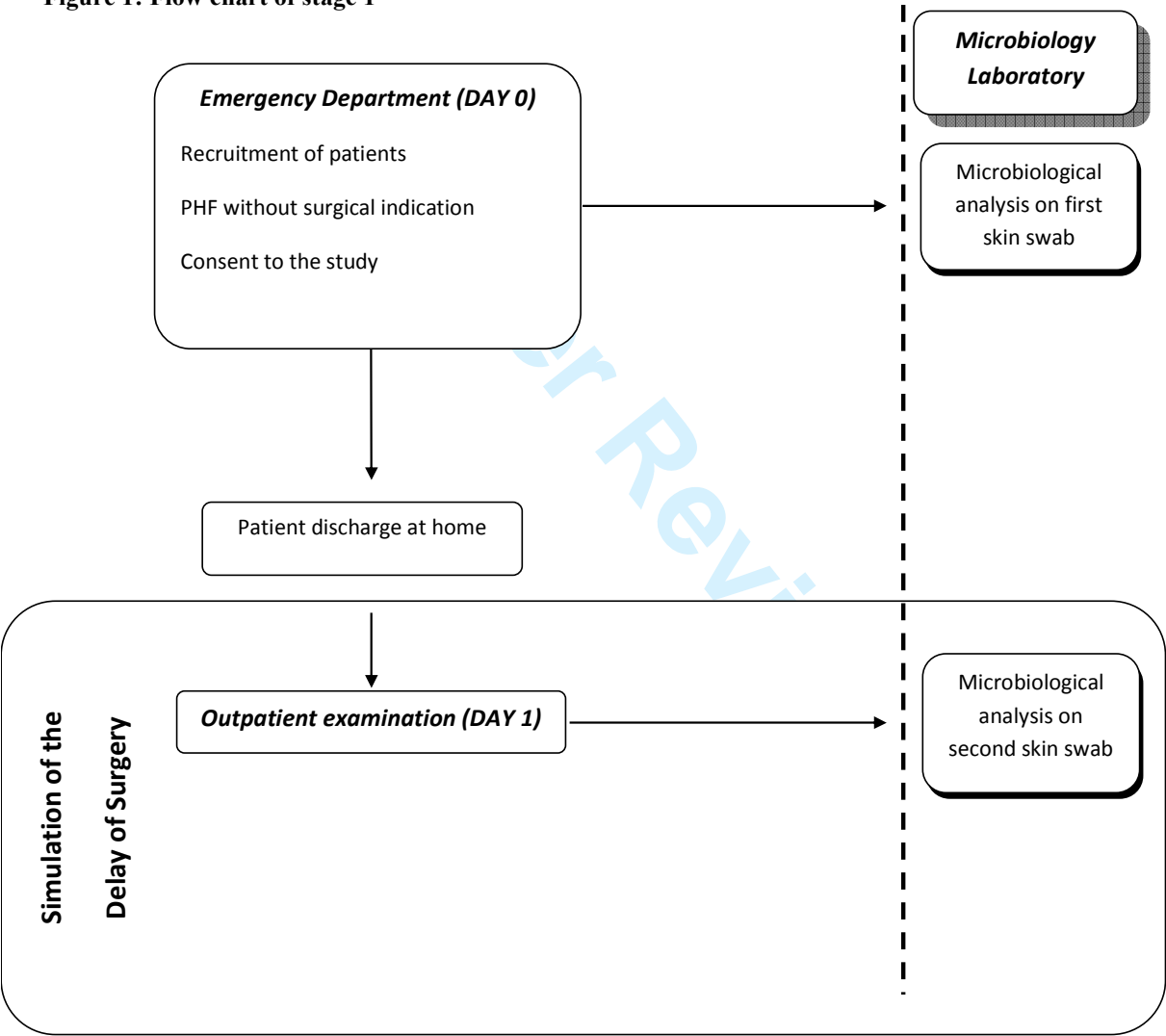
116 patient was scheduled to attend the outpatient clinic the day after the trauma (day 1, to

117 simulate an acute surgery) and the fifth day after the trauma (day 5, to simulate a delay of 5

118 days). In each of the two examinations, a skin culture swab was taken from the area of the

119 deltopectoral approach (Figure 1).

Figure 1: Flow chart of stage 1



Second stage.

In the second stage of the study, 20 consecutive patients affected by displaced proximal humeral fractures scheduled for surgical treatment were included. All the patients were recruited in the emergency room the day of the trauma, where the first skin culture swab was taken from the area of the deltopectoral approach. The patients were placed in a bandage. The second skin culture swab was collected the day of surgery in the operative room, immediately before skin preparation. The delay to surgery usually depended on the availability of CT scan, hospital bed and operative room. All the patients had an antibiotic prophylaxis immediately before surgery with a 2g dose of cefazolin. The patients were followed up at 1 week, 2 weeks, 4 weeks, 3 months, 6 months and 1 year after surgery for diagnosis of superficial or deep infection.

Bacterial isolation and identification

Skin swabs were immediately put into sterile culture swab tubes that contained Amies transport medium (Becton Dickinson Italia S.p.a., BD, Buccinasco, Milan, Italy) and brought within one hour to the Bacteriology and Mycology Laboratory of the Department of Public Health and Pediatrics, University of Torino. Serial 10-fold dilutions were prepared for each sample in saline solution (0.9% NaCl) so that the number of colony-forming units (C.F.U.)/mL was determined; 100 µL of each dilution were spread on various cultural media suitable for aerobic and anaerobic bacteria. The following media were used: Nutrient Agar (NA; Oxoid S.p.A., Milan) for aerobic bacteria; Mannitol Salt Agar (MSA; Merck Bracco, Milan for staphylococci; Schaedler Agar plus 5% blood (BD) for anaerobic bacteria.

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145 Plates were incubated for 24-48 hours at 37°C under aerobic conditions for aerobic bacteria
146 and for up 7 to 14 days at 37°C under strictly anaerobic conditions within an anaerobic system
147 (Gaspak EZ anaerobe pouch system kit, BD) for obligate and facultative anaerobe bacteria.
148 After incubation, the C.F.U. number -was recorded. All colonies with different morphologies,
149 colors, sizes, and hemolytic reactions were selected so that as many of the predominant
150 bacterial types as possible could be obtained. For morphologic analysis, Gram staining was
151 performed and cellular morphologies were determined by light microscopy. Studies of
152 enzymatic activities and fermentation of sugars were used to identify isolated facultative
153 anaerobic/aerobic microorganisms. These biochemical tests were performed with
154 commercially available API systems (BioMérieux, Rome, Italy) for aerobic bacteria (i.e. API
155 Staph, API 20NE) and for anaerobic bacteria (i.e. API 20A), according to the manufacturers'
156 instructions. For the count of the different bacterial species we used the higher number of
157 species measured in each media.
158 Statistical analysis. The differences between day 0, day 1, day 5 (stage 1) and between trauma
159 and surgery, were compared using the Wilcoxon test for paired samples. This test was
160 preferred after the Kolmogorov-Smirnov test for normality revealed a not-normal distribution
161 of the data.

Results:

First stage

Out of the 25 patients initially included in the first stage of the study, 5 were excluded because they did not attend one of the scheduled appointments therefore swab cultures could be collected. The remaining 20 patients formed the studied population.

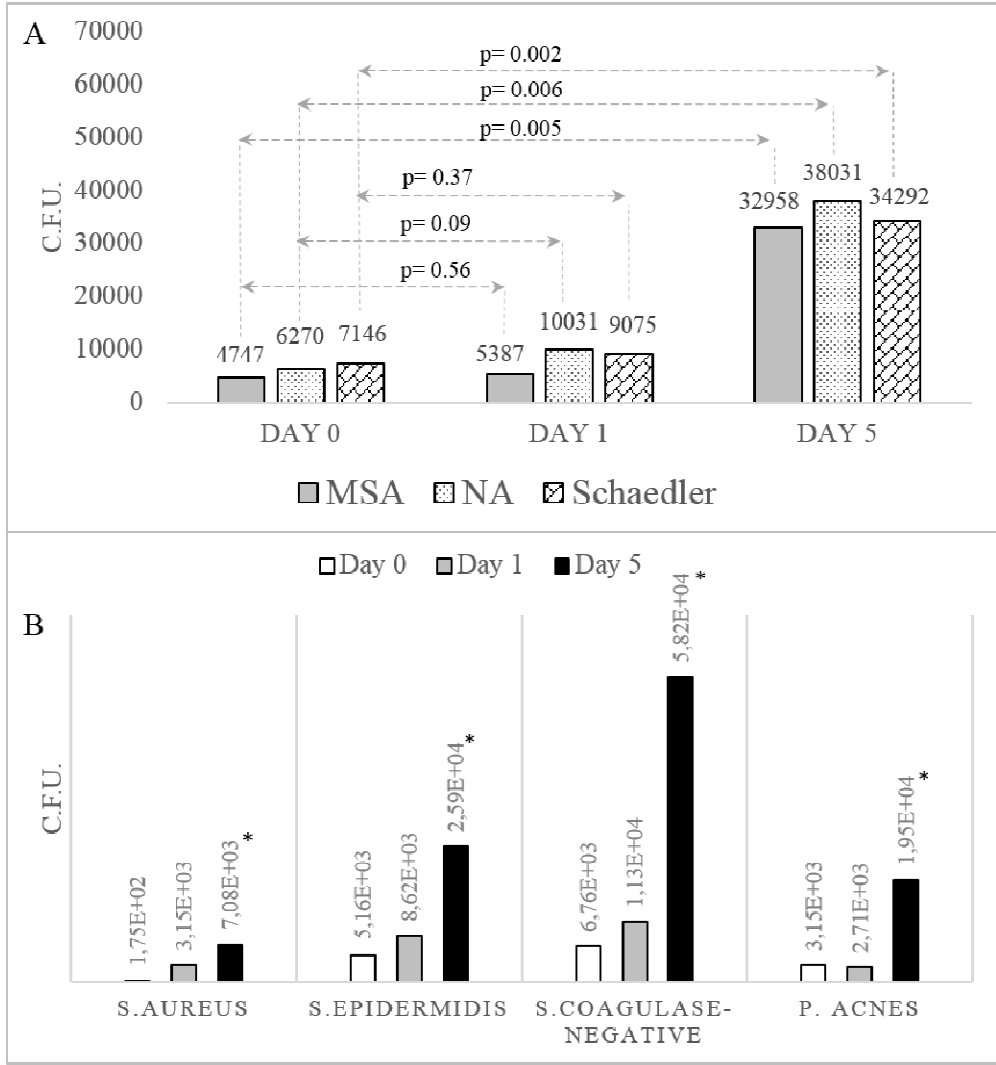
The average age of the patients was 67 years (range 50-85 years), 12 (60%) were female.

The number of bacteria on skin culture swabs increased over the course of the study, from day 0 to day 5, both considering the total number of C.F.U. on the individual cultural media (Figure 2A) and the C.F.U. of the individual species of bacteria (Figure 2B). The C.F.U. on MSA increased from 4.75×10^3 C.F.U. the day of trauma to 5.39×10^3 on day 1 ($p = 0.56$), to 3.30×10^4 on day 5 ($p = 0.005$). The C.F.U. on NA increased from 6.27×10^3 C.F.U. the day of trauma to 1.00×10^4 on day 1 ($p = 0.09$), to 3.80×10^4 on day 5 ($p = 0.006$). The C.F.U. on Schaedler Agar increased from 7.15×10^3 C.F.U. the day of trauma to 9.08×10^3 on day 1 ($p = 0.37$), to 3.43×10^4 on day 5 ($p = 0.002$).

Considering the single pathogen bacteria, 3 patients had a positive culture for *Staphylococcus aureus* the day of trauma and 7 patients on day 5 post trauma. Twelve patients had a positive culture for *Staphylococcus epidermidis* the day of trauma and 13 on day 5 post trauma. Eleven patients had a positive culture for *Propionibacterium acnes* the day of trauma and 13 on day 5 post trauma. *S. aureus* increased from 1.75×10^2 C.F.U. (day 0), to 3.15×10^3 C.F.U. on day 1 ($p = 0.14$), and to 7.08×10^3 C.F.U. on day 5 ($p = 0.04$). The *P. acnes* decreased from 3.15×10^3

C.F.U. (day 0), to 2.71×10^3 C.F.U. on day 1 ($p=0.05$), but increased to 1.95×10^4 C.F.U. on day 5 ($p=0.03$). The coagulase-negative Staphylococci (CoNS) increased from 6.76×10^3 C.F.U. on day 0, to 1.13×10^4 C.F.U. on day 1 ($p=0.1$), and to 5.82×10^4 C.F.U. on day 5 ($p=0.002$) (Figure 2B).

Figure 2A&B



Second stage

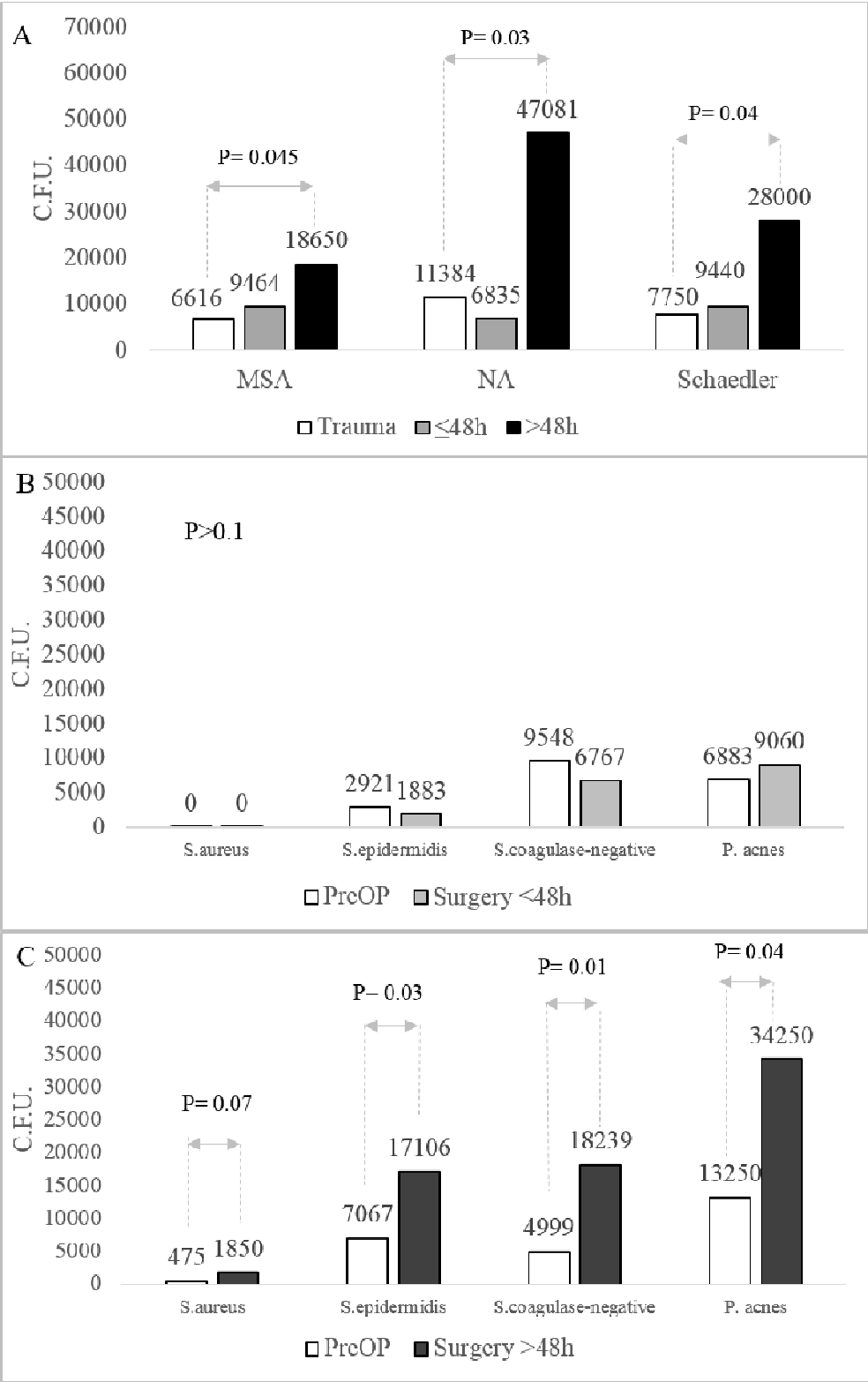
The 20 patients included in the second stage of the study underwent surgery at an average of 4.4 days from trauma (range 0 to 7 days). The average age of the patients was 65 years (range 45-90 years), 14 (70%) were female. Seventeen patients underwent a reduction and fixation

194 using an external fixator¹¹⁻¹³, 2 a reverse arthroplasty and one emiarthroplasty¹⁴. For
195 descriptive purpose patients were divided into two subgroups according to the delay to
196 surgery. In the first group patients underwent surgery within 2 days from trauma (9 patients,
197 average delayed 1.7 days), in the second group patients that underwent surgery after 2 days
198 from trauma (11 patients, average delay 6.4 days).

199 The data in Figure 3A,B,C confirm the data observed in the first stage of the study. A
200 bacterial increase was observed in the patients that underwent surgery after 2 days from
201 trauma. The change of bacteria between trauma to surgery, in the patients that underwent
202 surgery within 2 days from trauma, was not statistically significant ($p > 0.1$). The increase
203 from trauma to surgery was significant in the subgroup of patients that underwent surgery
204 after 2 days for all the variables ($p < 0.05$) except for the increase of *S. aureus* (Figure 3C).

205

Figure 3A,B,C



209 In the subgroup of 9 patients that underwent surgery within 2 days form trauma, one had a
210 positive culture for *S. aureus*, 5 patients had a positive culture for *S. epidermidis* the day of
211 trauma and at surgery, 8 patients had a positive culture for CoNS the day of trauma and at
212 surgery, 5 patients had a positive culture for *P. acnes* the day of trauma and at surgery.

213 In the subgroup of 11 patients that underwent surgery after 2 days form trauma, 4 had a
214 positive culture for *S. aureus* the day of trauma and 5 at surgery, 8 patients had a positive
215 culture for *S. epidermidis* the day of trauma and 10 at surgery, 9 patients had a positive culture
216 for CoNS the day of trauma and 10 at surgery, 5 patients had a positive culture for *P. acnes* the
217 day of trauma and 7 at surgery.

218 One patient (5%) had a diagnosis of deep infection 6 months after surgery (shoulder
219 hemiarthroplasty). He had persistent shoulder pain, radiographic signs of infection and
220 positive bone scan. An arthrocentesis was performed for identification of pathogen bacteria.
221 The culture was positive for *P. acnes*. This patient had a positive culture the day of trauma for
222 *P. acnes* (2.93×10^4 C.F.U.). He underwent surgery 5 days from trauma when the number of
223 *P. acnes* increased to 1.52×10^5 C.F.U.

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Discussion:

The aim of this study was to demonstrate if the delay to surgery increases the bacterial colonization of the shoulder skin at the level of the surgical approach. The outcomes confirm that the number of bacteria, including pathogen bacteria, increases on the skin surface during the time between trauma to surgery. Although this study clearly shows an increase of pathogen bacteria, this does not prove that the delay to surgery increases the rate of infection. The occurrence of infection is, in fact, multifactorial but we here analyzed only one of the potential factors. Other variables such as patient's immune status, type of antibiotic prophylaxis or type of skin preparation could affect the infection rate.

A recent study by Blonna *et al.* analyzed risk factors for acute infection after fixation for proximal humeral fracture¹⁰. This retrospective multicenter study analyzed 452 proximal humeral fractures with an infection rate of about 4%. The most common bacteria were *S. aureus* and CoNS. In the present study we showed that the number of *S. aureus* and CoNS increased significantly when the surgery is performed 5 days after trauma (in a simulated model) or after 2 days from trauma (in the "in vivo" model). Our report also evidenced that the *P. acnes* increased by 6 times 5 days after trauma. Interestingly the patient that, in our series, had a chronic *P. acnes* infection, underwent surgery 5 days after trauma and had an extremely high number of *P. acnes* at the level of deltopectoral approach.

Although these data alone do not prove the association between delay to surgery and infection rate, they strongly suggest that this topic deserves further studies. Recently the scientific community attention has been focused on *P. acnes*, since this bacterium is believed to be one of the major responsible of chronic infection in shoulder¹⁵⁻¹⁸.

249 In our series, the delay to surgery increased significantly the number of *P. acnes* and the effect
250 of the delay seemed to be more pronounced for this pathogen compared to other bacteria. The
251 local condition of the skin under the bandage could be responsible of this increase.

252 Other studies have investigated the bacteria responsible for post operative shoulders
253 infections. Athwal et al. reported 5 cases of acute shoulder infection in 259 patients that
254 underwent open reduction and fixation of proximal humeral fractures. Polimicrobial infection
255 was present in one case with the remaining cases presenting monomicrobial infection. CoNS
256 species were the most common microorganism isolated, infecting 3 of 5 patients whereas *P.*
257 *acnes* was isolated from 2 patient⁸.

258 Smith et al.¹⁹ analyzed early complications of proximal humeral fractures. Out of 22
259 hemiarthroplasty, 2 had a deep infection. One infection was due to *S. epidermidis* and the
260 other to both *S. aureus* and *P. acnes*. Both were treated with surgical debridement, intravenous
261 antibiotics, and retention of the components. Of the 82 shoulders that underwent surgery there
262 was one acute deep infection (*S. epidermidis*) treated at 3 weeks with 2 surgical debridements,
263 intravenous antibiotics, and retention of internal fixation with no recurrence of infection.
264 Furthermore in this group one patient had his plate broken without healing of the fracture site.
265 At the time of re-intervention he had a positive culture at the nonunion site (*P. acnes*)¹⁹.

266 The direct correlation between bacterial load and type at the level of the deltopectoral
267 approach and infection rate, although conceivable, has never been proved. However some
268 studies analyzed the effect of shoulder skin preparation on bacteria, suggesting that the
269 number of bacteria is proportional to the risk of infections¹⁹⁻²². Saltzman et al. showed that a
270 2% chlorhexidine gluconate and 70% isopropyl alcohol is more effective than povidone-
271 iodine in eliminating overall bacteria from the shoulder region²². The results of this "in vitro"

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study were furthermore confirmed by a clinical multicenter study that showed that washing the shoulder with 4% gluconate chlorhexidine and a subsequent preparation with iodopovidone, can significantly reduce the infection rate¹⁰.

This study has at least three main clear limitations. The first one is that the number of patients included is not enough to be sure that delay of surgery is the main risk factor for infection after surgical treatment for proximal humeral fracture. In this series among these patients only one had a diagnosis of deep infection. A multicenter study would be advisable. The second limitation is that the effect of the delay was tested only considering 5 days after trauma. However we decided to focus on the first 5 days after trauma because this is the most common clinical condition. We cannot exclude that longer delay could influence the type of bacteria in an unexpected way. In other districts there are evidences suggesting that delaying surgery after 7 days can reduce the infection rate. Blonna *et al.* showed a slight reduction of infection after proximal humeral fracture when surgery was performed after one week¹⁰. This aspect needs to be investigated in further studies.

The last limitation, already reported above, is that an increased number of bacteria is not synonymous of infection because other variables such as the type of skin preparation could affect the rate of infection. In an ongoing study, we are analyzing the effect of skin preparation in relationship with the number and type of bacteria before skin disinfection. This would represent another step forward in the prevention of infection.

294 In conclusion this study demonstrates that delaying the surgical treatment of a proximal
295 humeral fracture increases the number of skin pathogen bacteria above the deltopectoral
296 sulcus, potentially affecting the infection rate.

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301 The authors have no conflicts of interest to declare

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For Peer Review

Legends:

Figure 1: **Flow chart of stage 1 study**

PHF: Proximal Humeral Fracture

Figure 2A, B: **Bacterial increasing overtime in conservatively treated patients (stage 1)**

The number of bacteria on skin culture swabs increased over the course of the study, from day 0 to day 5, both considering the total number of C.F.U. on the individual culture media (A) and the C.F.U. of the individual bacterial species (Figure B).

C.F.U.= Colony Forming Unit

MSA= Mannitol Salt Agar

NA= Nutrient Agar

*= $p < 0.05$

Figure 3A, B, C: **Bacterial increasing overtime in surgically treated patients (stage 2)**

In the patients that underwent surgery within 2 days from trauma the change of bacteria was not statistically significant ($p < 0.1$, Figure A, B). An increase of bacteria was observed in the patients that underwent surgery after 2 days from trauma, both considering the total number of C.F.U. on the individual culture media (A, C).

C.F.U.= Colony Forming Unit

MSA= Mannitol Salt Agar

NA= Nutrient Agar